

09/4/96, 7/29

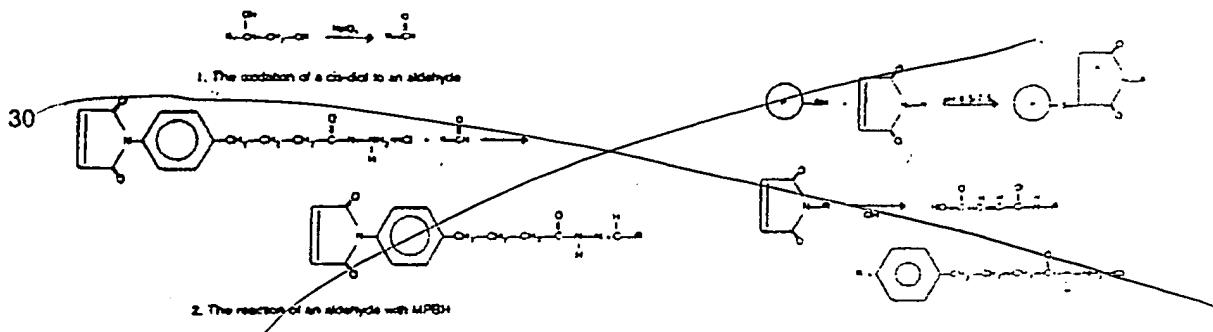
- 25 -

The neutralizing capacity of antisera can be quantitated by utilizing an assay which will detect single round of HIV-1 entry. Recombinant HIV-1 lacking *env* and possessing a CAT reporter gene are complemented in *trans* with various envelope glycoproteins, allowing infection of CD4-positive T-
5 cells. This assay can be used to determine the titer of homologous neutralization as well as the breadth of the neutralizing response by assessing the capacity of the antisera to cross-neutralize viruses with heterologous laboratory-adapted and primary envelope glycoproteins.

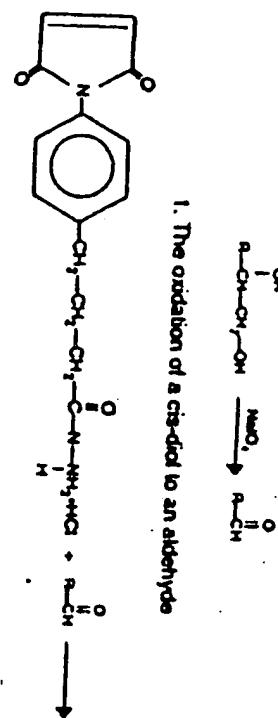
These selectively deglycosylated polypeptides should elicit antibodies
10 capable of neutralizing a broad range of laboratory-adapted strains better than wild-type glycoproteins. In generating an immune response, the presence of such a rage of antibodies will have a valuable role by significantly diminishing the acute viremia phase observed in most individuals soon after infection with HIV-1. Those glycoproteins containing
15 multiple glycosylation site mutations as opposed to the wild-type are preferred.

To derivatize the sugars distal from gp120 receptor binding regions, gp120 sugar residues containing vicinal hydroxyls will be oxidized to aldehydes using sodium meta-periodate (NaIO_4^- , Reaction 1). These
20 aldehydes can then be reacted with the hydrazide group on the heterobifunctional cross-linker MBPH (Pierce) to form hydrazone (Reaction 2).

The MBPH also contains a reactive melamide group, which is selective for sulphhydryls when the pH of the mixture is kept between 6.5 and
25 7.5 (Reaction 3). In *Drosophila* cells, we will produce recombinant murine C3d that has been genetically altered to remove its reactive group



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2. The reaction of an aldehyde with MPBH

